510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K042092

B. Purpose for Submission:

New Device

C. Measurand:

IgM class antibody to Epstein-Barr virus - viral capsid antigen (EBV-VCA)

D. Type of Test:

Multiplexed serology assay on flow cytometry platform

E. Applicant:

Zeus Scientific, Inc.

F. Proprietary and Established Names:

AtheNA Multi-Lyte® EBV VCA IgM Test System

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3235

2. Classification:

   Class I

3. Product code:

   LJN

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The Zeus Scientific, Inc. AtheNA Multi-Lyte® EBV VCA IgM Test System is a microparticle-based immunoassay intended for the qualitative detection of IgM class antibody to Epstein-Barr virus, viral capsid antigen in human serum. The test system is intended to be used for the laboratory diagnosis of EBV-associated infectious mononucleosis and provides epidemiological information on the diseases caused by Epstein-Barr virus.

2. Indication(s) for use:

   The Zeus Scientific, Inc. AtheNA Multi-Lyte® EBV VCA IgM Test System is for the laboratory diagnosis of EBV-associated infectious mononucleosis.

3. Special conditions for use statement(s):

   This device is for prescription use only

4. Special instrument requirements:

   AtheNA Multi-Lyte® System.

I. Device Description:

The AtheNA Multi-Lyte® EBV VCA IgM Test System is a microparticle bead-based immunoassay system for the qualitative detection of IgM antibody to EBV-VCA in human serum.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   EBV VCA IgM ELISA Test System

2. Predicate 510(k) number(s):

   K944449

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	For the qualitative detection of IgM class antibody to Epstein-Barr virus for the laboratory diagnosis of EBV-associated infectious mononucleosis	Same
Capture antigen	EBV VCA pg125	Same
Sample matrix	Serum	Same

Differences		
Item	Device	Predicate
Assay format	Flow cytometry immunoassay	Standard plate ELISA
Reporter conjugate	Phycoerythrin	Alkaline phosphatase
Solid Phase	Polystyrene microspheres	Polystyrene microwell plates

K. Standard/Guidance Document Referenced (if applicable):

Not Applicable

L. Test Principle:

Test sera are incubated with a multiplexed bead suspension which contains a mixture of distinguishable sets of polystyrene microspheres. Conjugated to the primary set of microspheres is the EBV viral capsid antigen. Three different calibration bead sets are also present in the multiplexed bead suspension so that a calibration curve is run with each individual sample. A final bead set is conjugated to affinity purified human IgM, which serves as a positive internal control for the reaction. When patient sera are added to the multiplexed bead suspension, patient antibodies specific for EBV-VCA are captured on the antigen-coated microspheres. Phycoerythrin (PE)-conjugated goat anti-human IgM is then added, which will bind to the patient IgM captured by EBV-VCA antigen. The bead suspension is then analyzed on the AtheNA Multi-Lyte instrument. PE signal can be determined for each individual bead set.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

a. *Precision/Reproducibility:*

Assay precision was evaluated at multiple sites as follows: Six samples were identified for use in the study based upon their activity on the AtheNA assay. Two samples were selected that were clearly negative, two that were clearly positive and two samples that were near the assay cut off. This panel of six serum samples were split into three aliquots each and tested at the three clinical sites. One each day of testing, each sample was diluted twice and then each dilution was run in quadruplicate, resulting in eight results per assay. This was performed on three days at each facility.

The summary of the precision study appears below:

AtheNA Multi-Lyte EBV VCA IgM Precision Summary

Panel Member	Mean U/mL	Site	Within Run Day 1 SD	Within Run Day 1 % CV	Within Run Day 2 SD	Within Run Day 2 % CV	Within Run Day 3 SD	Within Run Day 3 % CV	Between Day SD	Between Day % CV	Between Sites SD	Between Sites % CV
1	19	1	1.66	8.7%	2.51	13.2%	1.55	7.8%	1.91	9.8%	3.55	23.2%
2	15	1	1.16	8.2%	1.73	10.2%	1.16	7.9%	1.76	11.5%	2.04	12.7%
3	902	1	35.65	3.9%	52.15	5.7%	44.51	5.0%	45.48	5.0%	59.13	6.7%
4	435	1	26.39	5.9%	19.63	4.6%	19.78	4.6%	22.58	5.2%	37.69	9.0%
5	151	1	11.24	7.2%	10.91	7.6%	7.69	5.0%	10.69	7.1%	15.21	10.9%
6	192	1	13.81	7.2%	13.97	7.1%	10.01	5.4%	13.00	6.8%	17.49	9.7%
1	13	2	1.67	12.6%	1.85	16.1%	2.79	20.8%	2.13	16.9%		
2	16	2	1.04	6.0%	1.69	11.7%	2.75	17.3%	2.22	14.0%		
3	836	2	45.24	5.2%	46.84	5.5%	40.10	5.0%	51.80	6.2%		
4	391	2	35.43	8.7%	28.72	7.4%	40.09	10.6%	35.35	9.0%		
5	130	2	8.75	6.1%	5.45	4.5%	14.41	11.7%	14.18	10.9%		
6	167	2	10.56	5.8%	15.33	9.4%	15.34	9.7%	16.86	10.1%		
1	14	3	2.00	14.8%	1.07	7.9%	2.17	15.6%	1.74	12.8%		
2	17	3	1.77	10.6%	1.49	8.4%	2.00	11.8%	1.75	10.2%		
3	897	3	20.49	2.4%	47.06	5.1%	63.53	6.9%	56.76	6.3%		
4	427	3	33.20	8.5%	29.23	6.5%	28.63	6.5%	38.51	9.0%		
5	139	3	7.84	6.1%	14.82	10.0%	8.38	6.0%	12.82	9.2%		
6	181	3	9.02	5.3%	12.75	7.0%	6.79	3.5%	13.40	7.4%		

b. *Linearity/assay reportable range:*

Not Applicable. Assay is qualitative only.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Not Applicable. No widely recognized reference material (such as a WHO standard) available.

d. *Detection limit:*

Not Applicable. Limit of Detection can not be established without a widely recognized reference material.

e. Analytical specificity:

The AtheNA Multi-Lyte EBV VCA IgM test system was evaluated for potential cross reactivity to other antibodies. For this study, a total of 30 specimens were evaluated. Thirteen of the specimens were positive for IgM antibody to other infectious disease agents (Cytomegalovirus, Herpes Simplex Virus, Rubella and Toxoplasma). Of the thirteen specimens evaluated, none were reactive on the AtheNA Multi-Lyte EBV VCA IgM assay. Fourteen specimens were tested that possessed various autoantibodies to nuclear antigens. Of the fourteen specimens tested, none of them were reactive on the AtheNA Multi-Lyte EBV VCA IgM assay. Finally, four specimens were tested that were found to be RF IgM positive. All four specimens yielded invalid results on the AtheNA Multi-Lyte EBV VCA IgM assay. These specimens were invalidated by Intra-Well Calibration Technology®, since the non-specific bead contained in the multiplex bead suspension is designed to detect such activity and invalidate the specimens.

f. Assay cut-off:

The cutoff for this assay was originally set against a panel of 147 negative specimens from normal blood donors. It was assumed that this population should, in large part, be free of acute EBV infection and therefore display a low incidence of EBV VCA IgM antibody. These samples were tested and the mean fluorescence and standard deviation were determined for this population. The cutoff was set equal to the mean plus three times the standard deviation. Validation of this analytically set cutoff can be seen in the method comparison and clinical studies sections below (sections 2a and 3).

2. <u>Comparison studies:</u>

a. Method comparison with predicate device:

There were a total of 763 specimens tested. Of the 763 specimens tested, 693 were prospective specimens and 70 were retrospective specimens. The retrospective specimens were purchased as "expected acute" specimens based on clinical signs and symptoms of acute infectious mononucleosis. The actual EBV infection classification of both the prospective and retrospective specimens was determined as described below.

The EBV infection classification for each patient in the acute infection, no infection, past infection and indeterminate populations (763 patients total) was determined by a heterophile antibody latex agglutination assay, plus serological assessment using EBV marker profiles obtained from results of commercially available, FDA- approved ELISA reference assays. The serological assessment included the following 3 EBV markers: IgG antibody to Epstein–Barr virus viral capsid antigen (EBV VCA IgG), IgG antibody to Epstein–Barr virus nuclear

antigen 1 (EBNA-1 IgG), and IgM antibody to Epstein–Barr virus viral capsid antigen (EBV VCA IgM). The individual Athena Multi-Lyte EBV VCA IgM assay result was compared to the reference EBV VCA IgM assay result and to the patient classification. Each patient's EBV infection was classified based on the reactive (+)/nonreactive (-) patterns of the 3 EBV reference serological markers and the heterophile antibody assay. These patterns are presented in the following table:

EBV infection classification by reference assays:

EBV Classification	Prospective Specimen Group	Retrospective Specimen Group	Heterophile	VCA IgG	VCA IgM	EBNA-1 IgG
Acute infection	28	50	+	+	+	–
			+	–	+	–
			–	+	+	–
No infection	95	1	–	–	–	–
			n/a	–	–	–
Past infection	480	3	–	+	–	+
			n/a	+	–	+
Indeterminate	90	16	+	+	+	+
			+	+	–	+
			–	–	+	+
			–	+	+	+
			–	+	–	–
			–	–	+	–
			–	–	–	+
			n/a	+	–	–
Total:	693	70	+ = Reactive – = Nonreactive n/a = not available			

Note: When a reference assay result was equivocal, it was assumed to be nonreactive (-) for classification purposes.

Comparison of Results in Acute, No Infection, Past Infection, and Indeterminate Populations; AtheNA EBV VCA IgM Assay versus Comparative EBV VCA IgM ELISA Assay

(PROSPECTIVE SPECIMENS)

	Comparative Anti-EBV IgM Assay				Comparative Anti-EBV IgM Assay				Comparative Anti-EBV IgM Assay				
	Negative				Equivocal				Positive				
EBV Classification	AtheNA EBV VCA IgM Assay				AtheNA EBV VCA IgM Assay				AtheNA EBV VCA IgM Assay				
	Reactive N	Nonreactive N	Equivocal[1] N	Invalid N	Reactive N	Nonreactive N	Equivocal[1] N	Invalid N	Reactive N	Nonreactive N	Equivocal[1] N	Invalid N	Total N
Acute	0	0	0	0	0	0	0	0	24	1	0	3	28
No Infection	0	94	0	1	0	0	0	0	0	0	0	0	95
Past Infection	3	440	1	32	0	4	0	0	0	0	0	0	480
Indeterminate	2	41	0	2	0	0	0	0	18	22	2	3	90
Overall	5	575	1	35	0	4	0	0	42	23	2	6	693

[1] Equivocal results following repeat testing

Comparison of Results in Acute, No Infection, Past Infection, and Indeterminate Populations; AtheNA EBV VCA IgM Assay versus Comparative EBV VCA IgM ELISA Assay

(RETROPROSPECTIVE SPECIMENS – EXPECTED ACUTE)

	Comparative Anti-EBV IgM Assay				Comparative Anti-EBV IgM Assay				Comparative Anti-EBV IgM Assay				
	Negative				Equivocal				Positive				
EBV Classification	AtheNA EBV VCA IgM Assay				AtheNA EBV VCA IgM Assay				AtheNA EBV VCA IgM Assay				
	Reactive N	Nonreactive N	Equivocal[1] N	Invalid N	Reactive N	Nonreactive N	Equivocal[1] N	Invalid N	Reactive N	Nonreactive N	Equivocal[1] N	Invalid N	Total N
Acute	0	0	0	0	0	0	0	0	48	0	0	2	50
No Infection	0	1	0	0	0	0	0	0	0	0	0	0	1
Past Infection	0	2	0	0	1	0	0	0	0	0	0	0	3
Indeterminate	0	0	0	0	0	0	0	0	14	2	0	0	16
Overall	0	3	0	0	1	0	0	0	62	2	0	2	70

[1] Equivocal results following repeat testing

For purposes of percent agreement calculations, the Athena EBV VCA IgM equivocal results (n=3) were assigned to the opposite clinical interpretation than that of the comparative assay result. Likewise, the comparative assay equivocal results were assigned to the opposite clinical interpretation than that of the AtheNA EBV VCA IgM result. The percent agreement between the AtheNA EBV VCA IgM assay and the comparative EBV IgM ELISA assay are summarized in the following tables by specimen EBV classification:

Percent Agreement and Confidence Intervals by EBV Classification; AtheNA EBV VCA IgM Assay vs. EBV VCA IgM Reference ELISA Assay

Prospective Specimens:

EBV Classification	Positive Percent Agreement % (x/n)[a]	95% Exact Confidence Interval	Negative Percent Agreement % (x/n)[b]	95% Exact Confidence Interval
Acute	96.0%(24/25)	79.6% – 99.9%	N/A[c]	N/A
No infection	N/A	N/A	100%(94/94)	96.2% - 100%
Past infection	N/A	N/A	99.1%%(440/444)	97.7% - 99.7%
Indeterminate	42.9%(18/42)	27.7% - 59.0%	95.3%(41/43)	84.2% - 99.4%
Overall	62.7%(42/67)	50.0% - 74.2%	99.0%%(575/581)	97.8% - 99.6%

a x = the number of AtheNA EBV VCA IgM results that are confirmed positive in agreement with the reference EBV VCA IgM confirmed positive results; n = the total number of reference EBV VCA IgM results that are confirmed positive

b x = the number of AtheNA EBV VCA IgM results that are nonreactive in agreement with the reference EBV VCA IgM; n = the total number of reference EBV VCA IgM results that are nonreactive

c Agreement resulted in 0/0 specimens. In such cases, percent agreement and 95% confidence intervals could not be calculated.

Percent Agreement and Confidence Intervals by EBV Classification; AtheNA EBV VCA IgM Assay vs. EBV VCA IgM Reference ELISA Assay

Retrospective Samples - Expected Acute

EBV Classification	Positive Percent Agreement % (x/n)[a]	95% Exact Confidence Interval	Negative Percent Agreement % (x/n)[b]	95% Exact Confidence Interval
Acute	100%(48/48)	92.6% - 100%	N/A[c]	N/A
No infection	N/A	N/A	100%(1/1)	N/A
Past infection	N/A	N/A	100%(2/2)	15.8% - 100%
Indeterminate	87.5%(14/16)	61.7% - 98.4%	N/A	N/A
Overall	96.9%%(62/64)	89.2% - 99.6%	100%(3/3)	29.2% - 100%

a x = the number of AtheNA EBV VCA IgM results that are confirmed positive in agreement with the reference EBV VCA IgM confirmed positive results; n = the total number of reference EBV VCA IgM results that are confirmed positive

b x = the number of AtheNA EBV VCA IgM results that are nonreactive in agreement with the reference EBV VCA IgM; n = the total number of reference EBV VCA IgM results that are nonreactive

c Agreement resulted in 0/0 specimens. In such cases, percent agreement and 95% confidence intervals could not be calculated.

b. Matrix comparison:

Not Applicable. Serum is the only indicated matrix for the assay.

3. Clinical studies:

a. Clinical Sensitivity:

Not Applicable.

b. Clinical specificity:

Not Applicable.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

Not Applicable. Cutoff was determined analytically (see section 1f above).

5. Expected values/Reference range:
The clinical study for the product included a total of 693 prospectively collected specimens. Aside from the samples tested at Zeus Scientific, Inc., specimens were tested at three other facilities; a university medical center located in Eastern U.S. and two hospitals located in Northeastern U.S.

Of the 693 specimens tested, 412 included the age and sex of the patient. These included specimens tested at Zeus Scientific and the university medical center. The two hospitals did not include age/sex data with their test results. The Athena Multi-Lyte EBV VCA IgM results for these 412 specimens by age group and gender are summarized in the following table:

Age (years)	Gender	Reactive (n)	Non-Reactive (n)	Equivocal (n)	Invalid (n)	Total (n)
1-9	Female	0	6	0	1	7
	Male	2	3	0	0	5
	Overall	2	9	0	1	12
10-19	Female	17	32	0	0	49
	Male	8	21	1	0	30
	Overall	25	53	1	0	79
20-29	Female	23	31	0	3	57
	Male	18	18	0	0	36
	Overall	41	49	0	3	93
30-39	Female	3	30	0	1	34
	Male	3	29	0	1	33
	Overall	6	59	0	2	67
40-49	Female	0	37	0	3	40
	Male	1	17	0	4	22
	Overall	1	54	0	7	62
50-59	Female	1	27	0	1	29
	Male	3	13	0	4	20
	Overall	4	40	0	5	49
60-69	Female	0	14	0	5	19
	Male	0	17	0	0	17
	Overall	0	31	0	5	36
70+	Female	0	6	0	2	8
	Male	0	4	0	2	6
	Overall	0	10	0	4	14
Total	Female	44	183	0	16	243
	Male	35	122	1	11	169
	Overall	79	305	1	27	412

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

1. The submitted information in this premarket notification is complete and supports a substantial equivalence decision.